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Measurement of Free Magnesium in Blood, Serum and Plasma with an Ion-Sensitive Electrode

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Summary: The fraction of total magnesium bound to protein and other substances depends upon the pH. pH-dependency of ionized free magnesium (iMg^{2+}) in serum is expressed by the *Siggaard-Andersen* equation:

$$iMg^{2+}\ (pH) = iMg^{2+}\ (7.4) \cdot 10^{x\,(7.4\,-\,pH)}.$$

During preparation of serum or plasma, considerable pH changes occur which have to be corrected on the basis of the above mentioned equation. For pH correction of iMg^{2+}, $x < 0.1$ has so far been used. However, this is correct only for new Mg^{2+}-sensitive electrodes. During the lifetime of Mg^{2+} electrodes used in the "Microlyte Magnesium" (Kone Instruments, Finland) x increases and $x = 0.2$ was found to be a suitable approximation for most of the lifetime. By instantaneous iMg^{2+} measurements in whole blood samples pH changes and the uncertainty of x can be avoided. Dilution of blood by intravenous infusions decreases x nearly proportionally to the decrease of protein concentration in blood. Various methodological influences such as temperature and delay time before centrifugation, storage of serum and venous occlusion were studied. The circadian rhythm of iMg^{2+} was found to be considerably more pronounced than that of total Mg and was negatively correlated to changes of free fatty acids. To avoid variations of iMg^{2+} due to circadian changes, blood collection should be carried out between 6 and 10 a.m. The normal range of iMg^{2+} in blood of 179 healthy subjects was found to be between 0.46 and 0.60 mmol/l and the quotient of free and total Mg between 0.59 and 0.71. The accuracy of "Microlyte Magnesium" (Kone Instruments, Finland) is sufficient in a wide range of iMg^{2+}.

Introduction

Ion-selective electrodes for the measurement of K^+, Na^+ and Ca^{2+} have been used in clinical laboratories for many years (1–4). The latest development in this field permits the measurement of free Mg^{2+} in blood serum by Mg^{2+}-sensitive electrodes (5–7). This method is of clinical importance because the functionally active fraction of magnesium in serum is ionized free magnesium (iMg^{2+}) (8–12). Mg^{2+}-sensitive electrodes are constructed by incorporation of a Mg^{2+} exchanger (ETH 5220 or ETH 7025) in a PVC membrane (4). The Mg^{2+} exchanger is not specific for Mg^{2+} but also detects other cations according to the sequence: $Mg^{2+} > Ca^{2+} \gg Na^+ = K^+$ (6, 13).

Total serum Mg concentration in healthy subjects amounts to 0.89 mmol/l (range 0.7 to 1.0 mmol/l), 32% being bound to proteins (mainly albumin), 3% to phosphate, 4% to citrate and 6% to other substances, 55% is iMg^{2+} (0.5 mmol/l) as determined by indirect methods (14).

Therefore, the concentration of iMg^{2+} in serum is dependent upon the pH and albumin concentration (9). A

change of the pH, as it occurs during preparation of serum, requires correction of the measured iMg^{2+}.

The present paper is a contribution to the process of standardizing the clinical method of iMg^{2+} measurement using "Microlyte Magnesium" (Kone Instrument, Finland) (13).

Methods

Sampling

Silicone in test tubes etc. has to be avoided, since it seriously disturbs the action of Mg^{2+}-sensitive electrodes (7). Na^+- or Li^+-heparin is the preferable anticoagulant when iMg^{2+} is to be measured in plasma. The heparin concentration should be less than 20 000 units/l (7, 15). EDTA and citrate have to be avoided because of Mg^{2+} binding.

pH dependency

In the "Microlyte Magnesium" instrument a theoretical correction of pH-dependent changes of iMg^{2+} is integrated. This is based on the *Siggaard-Andersen* equation (16):

$$iMg^{2+}\ (pH) = iMg^{2+}\ (7.4) \cdot 10^{x(7.4 - pH)}.$$

First we determined the exponent x of this equation. For this purpose blood samples from healthy subjects and from critical care patients were collected using evacuated glass tubes without silicone. Serum was separated by centrifugation and the serum of each subject was divided into about ten 0.5 ml portions. To investigate the influence of dilution upon the exponent x, a solution containing NaCl (140 mmol/l), KCl (4 mmol/l), $CaCl_2$ (1.2 mmol/l) and $MgCl_2$ (0.5 mmol/l) was added to a fraction of the serum samples. The pH values of the serum samples were varied between 6.6 and 8.2 by adding gaseous CO_2 or by shaking with air for different periods (0.5–3 min). pH, iMg^{2+} and iCa^{2+} were measured using the "Microlyte Magnesium" instrument.

For each set of serum samples iMg^{2+} was plotted as a function of pH and the linear regression was calculated for the logarithmic form of the *Siggaard-Andersen* equation

$$\lg[iMg^{2+}\ (pH)] = \lg[iMg^{2+}\ (7.4)] + x(7.4 - pH).$$

This calculation results in the individual values of iMg^{2+} (7.4) at pH = 7.4 and the individual exponents x in the *Siggaard-Andersen* equation, which describe the dependency of iMg^{2+} upon pH. These measurements and calculations were carried out in dependency of the number of measurements during the lifetime of one electrode, in dependency of serum dilution and for 34 individual subjects.

Effect of ionized Ca^{2+}

To investigate the effect of iCa^{2+} on iMg^{2+} we used standards with various iMg^{2+} and iCa^{2+} or serum as indicated in the legends to figures 4 and 5.

Reproducibility

Blood samples were collected from 5 patients in plain 7 ml vacutainer tubes and tubes containing Na^+-heparin (concentration 10 000–20 000 units/l) before breakfast (between 8 and 9 a.m.). The pH was measured immediately in whole blood. Serum and plasma were separated between 20 and 60 min after blood collection. iMg^{2+} and pH of each sample were measured ten times and iMg^{2+} was corrected to the original pH of whole blood.

Influence of venous occlusion, temperature and delay time before centrifugation

Blood samples with and without venous occlusion were taken from 12 healthy subjects. These samples were divided into 3 aliquots which were kept at 4 °C, 25 °C and 37 °C respectively for 60 min before centrifugation. Other blood samples were divided into 4 aliquots and kept at 25 °C for 5, 30, 60 and 120 min respectively before centrifugation.

Storage of serum samples

Four serum samples (0.5 ml) from each subject were filled into plastic tubes and sealed under exclusion of air. Aliquots were stored at + 4 °C and −20 °C. Four weeks later, iMg^{2+} and pH were measured. iMg^{2+} was corrected for pH = 7.4, and the differences to the mean value of iMg^{2+} before storage, also corrected to pH = 7.4, were calculated.

Circadian rhythm

In order to study circadian changes of total magnesium, iMg^{2+} and free fatty acids, blood was taken every 3 hours for a 24 hour period from 7 healthy 18 to 55 year old individuals without a history of drug or vitamin use. Total Mg in serum was measured by atomic absorption spectrophotometry (Instrument Lab. 251) and free fatty acids as described in l.c. (17).

Normal range

Blood samples of 179 healthy volunteers were collected. Serum was separated within 1 h after blood collection and stored at + 4 °C for 2 to 4 weeks. iMg^{2+} was corrected for pH = 7.4.

Results

pH dependency of iMg^{2+} and iCa^{2+} and ageing of Mg^{2+}-sensitive electrodes

To determine pH dependency of iMg^{2+}, different series of measurements of iMg^{2+} and pH with about 10 serum samples each and pH variations between 6.6 and 8.2 were carried out.

The regression analysis of each of these series resulted in a constant – the logarithm of iMg^{2+} at pH 7.4 – and the slope x of the linear regression of the logarithm of iMg^{2+} (pH). The squared regression correlation coefficient r^2 yielded 0.94 to 1, indicating a high reliability of these measurements.

Results of iMg^{2+} (7.4) measurement did not change during the lifetime of the electrode. The slope x, however, was smallest during the first measurements, reached a higher value after about 10% of the lifetime and kept this value until about 90% of the lifetime of the Mg^{2+} electrode. During the last 10% of the electrodes' lifetime the slope x increased considerably (fig. 1).

During the first measurements of iMg^{2+} and pH with a new electrode the slope amounted to x = 0.076 which is very near to the incorporated pH correction of the "Microlyte Magnesium" of x = 0.07. The slope

x = 0.07 would lead to a decrease of iMg^{2+} from 0.550 at pH = 7.4 to 0.516 at pH = 7.8.

During the last period of the lifetime of the electrode the pH dependency of iMg^{2+} yielded x = 0.32. With this slope, an increase of pH from 7.4 to 7.8 would cause a decrease of iMg^{2+} from 0.55 to 0.41. The pH dependency of the iCa^{2+} electrodes did not change systematically during their lifetime. According to figure 1, the pH dependency of a Mg^{2+} electrode is constant throughout the main part of its lifetime, i. e. after about 100 measurements to about 90% of lifetime. This normal slope is called x_0 Mg.

x_0 Mg of two different electrodes was measured with 24 sets of about 10 serum samples each from at total of 24 healthy subjects. With a third electrode x_0 Mg in serum of 12 patients was measured. The results are summarized in table 1.

Using the first electrode we found a mean slope of x_0 Mg = 0.25 ± 0.03 in the serum of healthy subjects. In the serum of intensive care patients we found a mean slope of x_0 Mg = 0.11 ± 0.04 with a second electrode. A third electrode had a mean slope of x_0 Mg = 0.17 ± 0.01 when serum of healthy subjects was used. The pH dependency for iCa^{2+} was measured parallel to the dependency of iMg^{2+} and found to have a slope of x_0 Ca = 0.18 ± 0.01 which is almost identical to x_0 Mg when measured with the same electrode 3.

The errors caused by a pH correction using a slope of x_0 Mg = 0.2 and an individual deviation of x_0 Mg of ± 0.1 (x_0 Mg: 0.1 and 0.3) are shown in figure 2 as a function of the deviation of pH (Δ pH) from the original value.

Influence of serum dilution upon the pH dependency of iMg^{2+}

The dilution of serum (e. g. during infusion) decreased the slopes of the pH dependency of iMg^{2+} and iCa^{2+}.

Tab. 1 Results of regression analysis of iMg^{2+} and pH measurements in serum of healthy subjects and patients.
iMg^{2+} (7.4), iCa^{2+} (7.4), x_0 Mg and x_0 Ca from regression analysis using the logarithmic form of the *Siggaard-Andersen* equation:

$$\lg[Mg^{2+}\ (pH)] = \lg[Mg^{2+}\ (7.4)] + x(7.4 - pH)$$

r^2: Squared multiple correlation coefficient.

		x_0 Mg	iMg^{2+} (7.4) (mmol/l)	r^2
Electrode 1				
Healthy subjects	Mean	0.25	0.55	0.99
n = 10	SD	0.03	0.04	0.01
	Max.	0.29	0.61	1.00
	Min.	0.21	0.49	0.96
Electrode 2				
Patients	Mean	0.11	0.63	0.97
n = 12	SD	0.04	0.07	0.02
	Max.	0.21	0.80	0.99
	Min.	0.05	0.54	0.94
Electrode 3				
Healthy subjects	Mean	0.17	0.60	0.98
n = 12	SD	0.01	0.04	0.01
	Max.	0.19	0.69	0.99
	Min.	0.16	0.54	0.96
		x_0 Ca	iCa^{2+} (7.4) (mmol/l)	r^2
	Mean	0.18	1.28	0.98
	SD	0.01	0.03	0.01
	Max.	0.20	1.33	0.99
	Min.	0.16	1.25	0.97

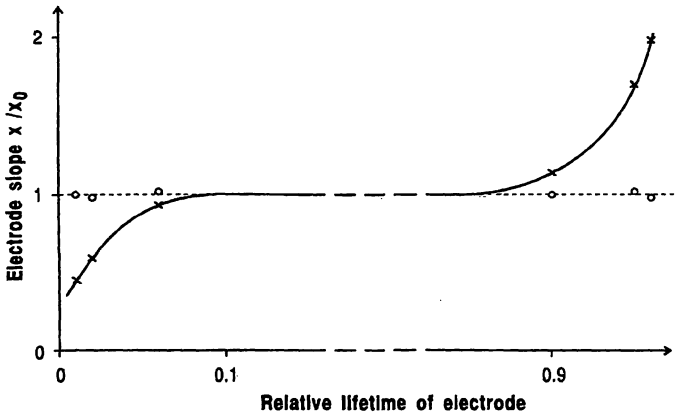

Fig. 1 Dependency of the relative changes of the slope x/x_0 at the beginning and the end of the lifetime of Mg^{2+} and Ca^{2+}-sensitive electrodes.
x_0 = slope in the middle of the electrode lifetime.
Total lifetime was defined as 1.
x–x Mg; o---o: Ca.

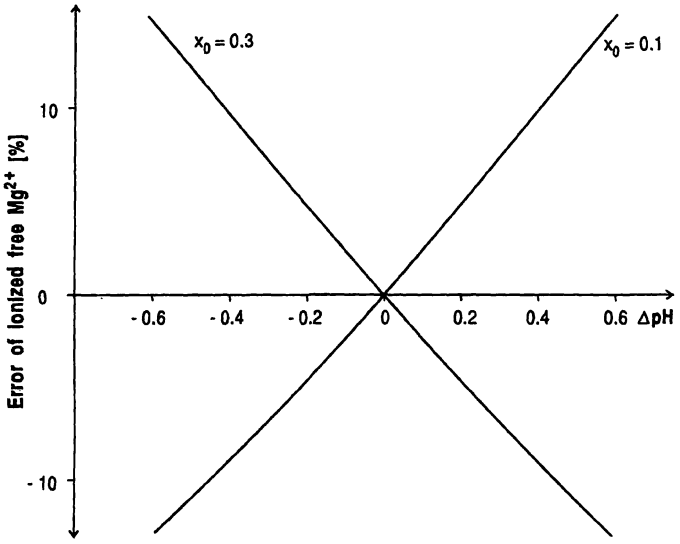

Fig. 2 Error of iMg^{2+} as a function of the pH variation (Δ pH) caused by pH correction with slope x_0 Mg = 0.2 when the correct slope is x_0 Mg = 0.1 or x_0 Mg = 0.03.

The results are shown in figure 3. The relative decrease of the slope was similar to the relative dilution of serum.

Without quantifying the amount of serum dilution, iMg^{2+} measurements in blood of patients treated with diverse infusion therapies can be carried out only under the condition that the pH at the time of iMg^{2+} measurement does not deviate by more than ± 0.4 from the original value. Under this condition the maximal error due to pH correction is in the range of −9% to + 15%, assuming a variability of slopes between 0.05 and 0.21 (see tab. 1).

Effects of iMg^{2+} and iCa^{2+} on the measurement of iMg^{2+}

To study the effect of iCa^{2+} on iMg^{2+}, we increased iCa^{2+} in blood serum which was diluted 1 : 2 with a solution containing 140 mmol/l NaCl, 5 mmol/l KCl, 0.5 mmol/l $MgCl_2$ and 6 mmol/l Hepes (pH = 7.4) by addition of up to 25 µl of 100 mmol/l $CaCl_2$ solution to 1000 µl diluted serum.

Since the ionic strength and activity coefficients of the diluted serum were not changed significantly by the addition of $CaCl_2$ solution, the software incorporated in the "Microlyte Magnesium" could be applied to measure iMg^{2+}. Moreover in figure 4 the quotient of iMg^{2+} (measurement) over iMg^{2+} (concentration) is given.

Figure 4 demonstrates that the influence of iCa^{2+} upon iMg^{2+} is negligible for iMg^{2+} measurements in serum.

Figure 5 shows that the relative iMg^{2+} [iMg^{2+} (measurement)/iMg^{2+} (concentration)] measured in aqueous solution is independent of iMg^{2+}.

Reproducibility

The relative difference from the mean value for each individual value of iMg^{2+} for 10 iMg^{2+} measurements was calculated.

In table 2 the standard deviation (SD) of Δ represents the mean relative error of reproducibility. The mean relative error for iMg^{2+} measurement in serum (2.4%) was slightly greater than in whole blood (1.7%). In whole blood the maximal and minimal relative differences were also smaller than in plasma and in serum.

Venous occlusion, temperature and delay time before centrifugation

Venous occlusion resulted in a non-significant increase of iMg^{2+} (7.4) by 0.5% (data not shown).

Keeping blood for 1 hour at increasing temperatures from 4 °C to 37 °C before centrifugation decreases iMg^{2+} (7.4): Storage at 25 °C leads to a 0.8% decrease and at 37 °C to a 2.7% decrease of iMg^{2+} (tab. 3).

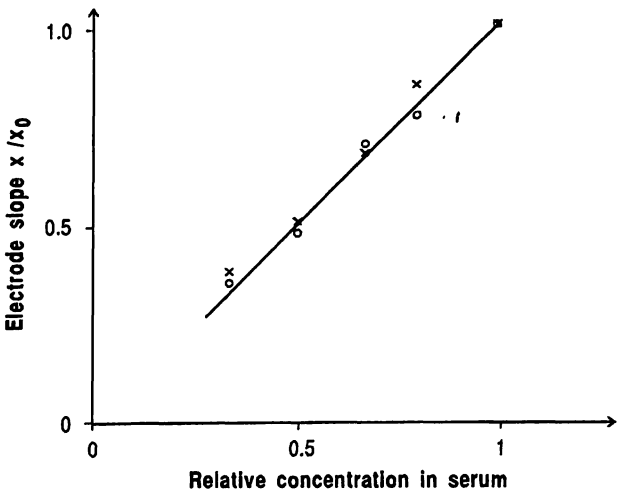

Fig. 3 Dependency of x/x_o on the dilution of blood serum.
1: undiluted, other values: serum diluted with a solution containing: 140 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l $CaCl_2$, 0.5 mmol/l $MgCl_2$.
x: Mg; o: Ca.

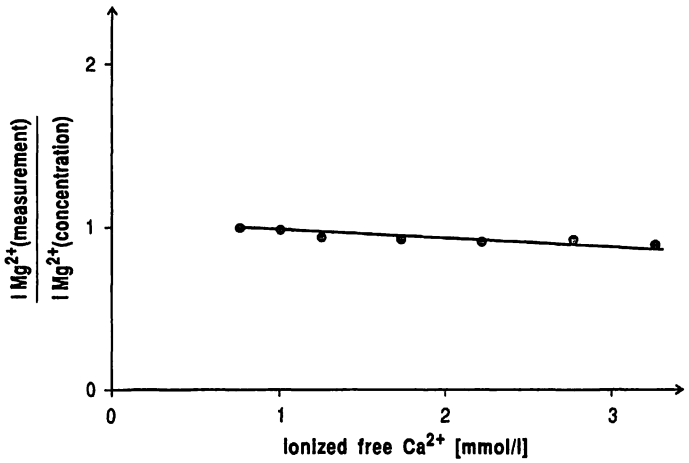

Fig. 4 Quotient of measured iMg^{2+} (iMg^{2+} measurement) divided by the iMg^{2+}-concentration) in diluted blood serum with the addition of $CaCl_2$ as a function of the measured iCa^{2+}. The quotient iMg^{2+} (measurement)/iMg^{2+} (concentration) without additional $CaCl_2$ was taken as 1.

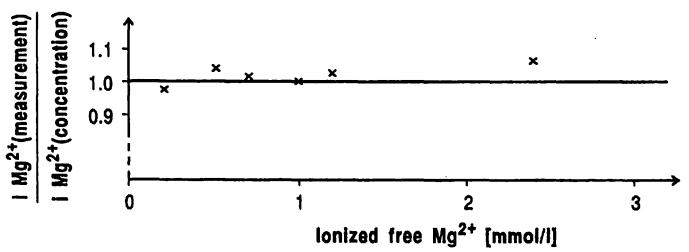

Fig. 5 Quotient of iMg^{2+} (measurement) divided by the added Mg^{2+} concentration (iMg^{2+}-concentration) in an aqueous solution containing 0.8 mmol/l $CaCl_2$, 140 mmol/l NaCl, 5 mmol/l KCl as a function of iMg^{2+}.

Keeping blood at 25 °C for various periods of time before centrifugation yielded no significant effect on iMg^{2+} (7.4) between 5 min and 1 hour. After 2 hours, a slight but significant decrease of iMg^{2+} (7.4) was detectable (tab. 4).

These results show that blood may be kept at room temperature (25 °C) for up to 1 hour before centrifugation. If centrifugation has to be delayed for more than 1 hour, blood samples should be kept in ice water.

Storage of serum samples

The relative differences in iMg^{2+} (7.4) after 4 weeks of storage in sealed plastic tubes at −20 °C and + 4 °C are given in table 5. Samples with a pH below 7.1 and above 7.8 after storage were excluded. The results show that storage at 4 °C is preferable and that the maximal error is 10%.

Circadian rhythm

The highest concentrations of iMg^{2+} and total Mg are observed at 9 a. m. and the minimum at 3 p. m. (fig. 6). There is a significant circadian variation of iMg^{2+} (max. difference approx. 10%). Total Mg did not change significantly (max. difference approx. 4%).

Free fatty acids showed a pronounced circadian rhythm. There was a negative correlation between free fatty acids and iMg^{2+}. These results indicate that free fatty acids in serum can bind Mg^{2+}, thus reducing iMg^{2+} up to 10%. Therefore, blood collection should be carried out between 6 and 10 a. m.

Reference range

Total Mg in serum of 179 healthy subjects ranged between 0.73 and 0.94 mmol/l and was identical to values

Tab. 2 Multiple determination (10 x) of iMg^{2+} in whole blood, plasma and serum of 5 patients (50 measurements each) (min. and max. differences to mean value in % and SD in %).

	Ionized Mg^{2+}		
	Whole blood Δ (%)	Plasma Δ (%)	Serum Δ (%)
min.	−5.8	−3.2	−7.6
max.	2.8	7.7	3.5
SD	1.7	1.8	2.4

Tab. 3 Effect of blood sample temperature before centrifugation on iMg^{2+}. Mean relative differences (Δ (%)) and SD of iMg^{2+} at various temperatures related to values at 4 °C, for example:

Δ (%) (25 °C) = [iMg^{2+} (25 °C) − iMg^{2+} (4 °C)] · 100/iMg^{2+} (4 °C).

Δ (%) (25 °C): −0.8	SD: 5.7	n: 11
Δ (%) (37 °C): −2.7	SD: 3.5	n: 11

Tab. 4 Mean relative differences (Δ (%)) and SD of iMg^{2+} when centrifugation was delayed, related to the values of 5 min.

Δ (%) (30 min) = iMg^{2+} (30 min) − iMg^{2+} (5 min)] · 100%/iMg^{2+} (5 min).

Δ (%) (30 min): −0.5	SD: 6.3	n: 11
Δ (%) (60 min): +0.1	SD: 5.6	n: 12
Δ (%) (120 min): −3.6	SD: 4.4	n: 12

Tab. 5 Relative differences (Δ (%)) of iMg^{2+} (mean values and SD) after 4 weeks of storage at two different temperatures. (pH: 7.1−7.8).

Δ (%) (−20 °C): 8.4	SD: 7.5	max.: 28	n: 13
Δ (%) (+4 °C): −1.2	SD: 5.4	max.: 10	n: 16

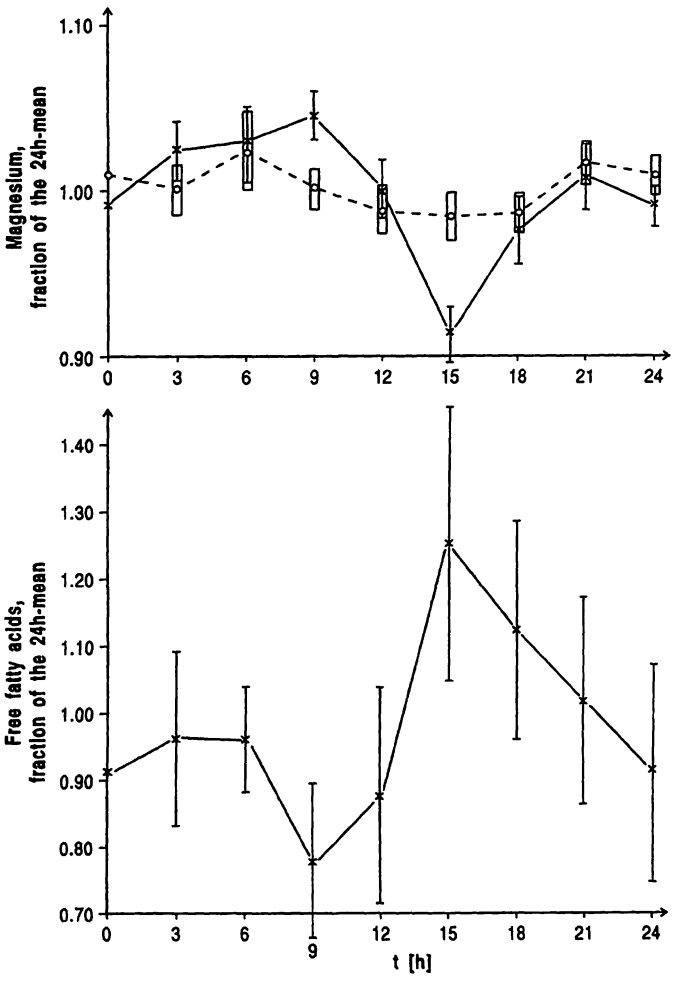

Fig. 6 Circadian rhythm of total Mg and iMg^{2+} (------: Mg total; ——: iMg^{2+}) and circadian rhythm of free fatty acids in serum of 7 healthy persons. Mean ± SD; 100% is 24 hours mean.

Tab. 6 Distribution of total Mg, iMg^{2+} and the quotient $Q = iMg^{2+}/Mg_{total}$ in serum of 179 healthy persons (hinge: 25% of distribution).

	Mg_{total} (mmol/l)	iMg^{2+} (mmol/l)	Q
Minimum	0.54	0.40	0.54
Lower hinge	0.80	0.52	0.62
Median	0.85	0.55	0.64
Upper hinge	0.88	0.57	0.67
Maximum	1.00	0.66	0.78

of the literature (9, 19, 20). iMg^{2+} in normal serum ranged between 0.46 and 0.60 mmol/l. In both ranges the upper and the lower 5% of the values were omitted. The quotient of free and total Mg ranged between 0.59 and 0.71, the median being 0.64 (tab. 6).

Discussion

Our results show that pH correction for iMg^{2+} measured by Mg^{2+} electrodes is far more complicated than for iCa^{2+}. The low slope of iMg^{2+} as a function of pH measured with a new electrode was erroneously taken as valid for the whole lifetime of Mg^{2+} electrodes in the Kone "Microlyte Magnesium". The presented results quantify the resulting error.

Since pH changes in whole blood samples are negligible within a few minutes after blood collection, iMg^{2+} measurements in whole blood avoid the problem of pH correction of iMg^{2+}. The disadvantage of this method is a decreased lifetime of the Mg^{2+} electrode when used in whole blood as compared to serum or plasma. This effect was minimized by introducing an automatic application of the "Maintenance" solution in the KONE "Microlyte Magnesium" and additionally a periodic application of the KONE "washing" solution by the user.

Errors of iMg^{2+} measurements in serum and plasma of more than ± 10% due to pH correction are avoided if the first 20 measurements in blood serum with a new Mg^{2+} electrode are discarded and if the accepted pH range of serum and plasma is limited to $7.0 < pH < 7.8$ and the pH correction is carried out using a slope $x = 0.2$. Using a slope of $x = 0.07$, a pH correction was valid in a very narrow pH range of 7.2 to 7.6 (24). Precise pH corrections in a pH range from 6.6 to 8.2 are possible if the first 100 measurements of a new Mg electrode are discarded and the individual slope x_o of the iMg^{2+} dependency upon pH is determined for the individual electrode. For the pH correction of patients' serum during or after an infusion therapy the degree of serum dilution has to be determined and with that the correct slope for pH correction of iMg^{2+} has to be estimated. This complicated procedure can be avoided by measuring iMg^{2+} in whole blood samples. If this is not possible and the serum dilution is unknown, a pH correction within a range of $7.0 < pH < 7.7$ using a slope x_o Mg = 0.11 would lead to errors of iMg^{2+} between −10% and + 15%.

In agreement with l. c. (15) we found that the precision of iMg^{2+} measurement by the "Microlyte Magnesium" is sufficient in a wide range of Mg^{2+} and Ca^{2+} concentrations. Independency of iMg^{2+} with varying iCa^{2+} was found in protein-containing samples.

The results of multiple determinations of one serum, plasma and whole blood sample demonstrate the precision of the method: the maximal relative difference of iMg^{2+} was 7.7% (tab. 2) and SD less than 3%. Therefore, accuracy and reproducibility of the "Microlyte Magnesium" is satisfactory and the method is recommendable for the easy and quick measurement of iMg^{2+} in whole blood. In order to measure iMg^{2+} in serum and plasma a more complicated procedure, such as has been described above, is necessary to maintain accuracy.

Our results on sample handling for iMg^{2+} measurements add some useful information to the literature on this subject (6, 24, 25). Serum of healthy subjects may be stored for at least 4 weeks at 4 °C. The error will not exceed 10% if only samples within a pH-range from 7.1 to 7.8 after storage are taken into account.

The normal range of free magnesium was 0.46 to 0.60 mmol/l which seems to be realistic. The mean value of the quotient of free and total Mg was 0.64. Other authors found 0.71 and 0.76 (15, 21). Indirect methods gave a result of 0.55 (14). Our results and the results of others (15, 22, 23) have shown that iMg^{2+} is higher than has been suggested so far by indirect methods.

Similar discrepancies were found with iCa^{2+} when measured by Ca^{2+}-sensitive electrodes in plasma and in ultrafiltrates. This effect was due to the *Donnan* effect which in the case of iCa^{2+} amounted to 8% (26).

The most significant result of this study was the circadian variation of iMg^{2+} which can cause a difference up to 10% without a significant alteration in total Mg. There was a significant negative correlation between iMg^{2+} and free fatty acids, indicating that Mg^{2+} is bound to free fatty acids, thus reducing iMg^{2+}.

Finally, it should be stated that the determination of iMg^{2+} is a valuable method to diagnose hypomagnesemic conditions which can occur even at normal values of total Mg in serum (18, 24).

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